

Article Addendum

Conservation and differences of the Min system in the chloroplast and bacterial division site placement

Hiromitsu Nakanishi,* Kenji Suzuki, Yukihiro Kabeya, Kumiko Okazaki and Shin-ya Miyagishima

Initiative Research Program; Advanced Science Institute; RIKEN; Wako, Saitama Japan

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Chloroplasts are descended from a cyanobacterial endosymbiont and divide by binary fission. Reminiscent of the process in their bacterial ancestor, chloroplast division involves a part of cyanobacteria-derived division machineries in addition to those acquired during chloroplast evolution.^{1,2} In both bacterial and chloroplast division, formation of the FtsZ ring at the mid position is required for subsequent constriction and fission at the mid division site.¹⁻⁴ As in bacteria, positioning of the FtsZ ring at the mid-chloroplast is mediated by the Min system.^{1,2} Recently, we identified the MCD1 protein, a plant-specific component of the Min system in *Arabidopsis thaliana* chloroplasts.⁵ Unlike other division components that have been acquired after endosymbiosis and function outside of the chloroplasts (i.e., in/on the outer envelope membrane),⁶⁻⁹ MCD1 functions inside the chloroplast. Since we already discussed about the function and significance of MCD1 as a division component of plant origin,⁵ here we focus on and discuss about the diversity and evolution of the Min system.

Components of the Min System in *Escherichia coli* and *Bacillus subtilis*

Bacterial cells usually divided binary division by constriction at the mid-cell position. The formation of a ring structure by polymerization of the tubulin-like protein FtsZ is the first known event at the division site, and it initiates the recruitment of the other proteins that comprise the bacterial division complex. The placement of the FtsZ ring at the mid-cell position is partly mediated by the Min system, which prevents FtsZ ring formation other than at

the mid-cell position.^{3,4} A large number of genome projects have revealed both partial conservation and also certain differences in the composition of the Min system in diverse bacterial lineages.³

The Min system has been studied extensively in both the proteobacterium (Gram-negative) *Escherichia coli* and firmicute (Gram-positive) *Bacillus subtilis*.^{3,4} In both organisms, MinC inhibits FtsZ ring formation, and MinD is a membrane-associated protein which recruits MinC to the membrane.¹⁰⁻¹³ The MinCD complex inhibits division other than at the mid-cell position, while allowing division at the mid-cell position.

Despite the conservative function of the MinCD complex, the topological specificity of the MinCD is regulated differently in *E. coli* and *B. subtilis*. In *E. coli* MinE sweeps the MinCD complex from one pole to the other so that MinCD undergoes subsequent pole-to-pole oscillation (Fig. 1). The time-averaged concentration of MinCD is maintained at a high level near the cell poles, and at a low level mid-cell. As a result, division is prevented at the cell poles and is only allowed at sites mid-cell.^{14,15} On the other hand, in *B. subtilis*, which does not have MinE, DivIVA recruits MinCD at the cell poles so that the FtsZ ring forms mid-cell. In *B. subtilis*, MinCD also localizes at the division site after FtsZ ring formation (Fig. 1),¹⁶⁻¹⁸ but the significance of the division-site localization is still unclear.

Components of the Min System in Cyanobacteria and Chloroplasts

Chloroplasts evolved from a cyanobacterial ancestor that was engulfed and enslaved by a eukaryotic host cell. Reminiscent of their bacterial ancestor, chloroplasts divide by binary division. Studies have shown that the chloroplast division involves FtsZ, MinD and MinE, genes which have been transferred from the engulfed cyanobacterium to the plant nuclear genome.^{1,2} Although the exact action of the Min system in cyanobacteria and chloroplasts has not been clarified, database searches and analyses of gene disruptants revealed that MinC, MinD, MinE and DivIVA-like protein are involved in cyanobacterial cell division (Fig. 1).^{19,20} Therefore, unlike the case in *B. subtilis* and *E. coli*, both MinE and DivIVA participate in cyanobacterial cell division.

Of the MinC, MinD, MinE and DivIVA-like proteins which are conserved in cyanobacteria, cyanobacteria-descended MinD and MinE are found in the genomes of green algae and land plants

*Correspondence to: Hiromitsu Nakanishi; Advanced Science Institute, RIKEN; 2-1 Hirosawa; Wako, Saitama 351-0198 Japan; Tel.: +81.48.467.9332; Fax: +81.48.467.9329; Email: h_nakanishi@riken.jp

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(Fig. 1).^{1,2} In addition, both have been shown to regulate the positioning of the FtsZ ring in the chloroplasts of *A. thaliana*^{21,22} and *Chlamydomonas reinhardtii*.²³ Putative MinC is evident in green algae (e.g., gi:145340725) and the moss *Physcomitrella patens* (gi:168012958) (Fig. 1),² although the function has yet to be characterized. These sequences are most closely related to those of cyanobacterial MinC, but missing in other land plant genomes, suggesting that MinC has been lost during the course of land plant evolution (Fig. 1). On the other hand, no DivIVA homolog has been found in the available plant and algal genome data, suggesting that DivIVA had been lost at an earlier stage of chloroplast evolution (Fig. 1).

Chloroplast-Specific Components of the Min System

In addition to MinD and MinE, studies on the *A. thaliana* chloroplast division mutants, *arc3*,^{24,25} and *mcd1*,⁵ have identified additional proteins that regulate FtsZ ring positioning in concert with MinD and MinE. The ARC3 protein is composed of an incomplete FtsZ-like domain, a middle domain with no recognizable sequence motifs, and a domain with partial similarity to phosphatidylinositol-4-phosphate 5-kinase (PIP5K). The PIP5K-like region bears membrane occupation and recognition nexus (MORN) motifs, and is known to bind to membranes. ARC3 is reported to localize at the division site and poles of chloroplasts (Fig. 1).^{24,25} Similar to the *A. thaliana minD* mutant, *arc3* mutation results in asymmetric chloroplast division and the formation of multiple FtsZ rings in single chloroplasts, indicative of a defect in FtsZ ring positioning.²⁴⁻²⁶ Similarly to bacterial MinC, ARC3 directly interacts with MinD, MinE and FtsZ, and overexpression of ARC3 inhibits FtsZ ring formation. Therefore, it is suggested that ARC3 fulfills the role of MinC, which is absent in land plants.^{1,2,25}

MCD1 spans the inner envelope membrane and bears a coiled-coil motif on the stromal side. Similar to the *minD* and *arc3* mutants, *mcd1* mutation results in asymmetric chloroplast division and the formation of multiple FtsZ rings in single chloroplasts.⁵ MCD1 directly interact with MinD, recruiting MinD to the chloroplast division site and the punctate structures dispersed on the inner envelope (Fig. 1).⁵ Like MCD1, DivIVA bears coiled-coil motifs and recruits MinCD, but the recruitment requires another component, MinJ, in *B. subtilis*,^{27,28} and there are no similarities between MCD1 and DivIVA in terms of amino acid sequence.

ARC3 is found in the genomes of both green algae and land plants, whereas MCD1 is specific to land plants, suggesting that ARC3 became integrated into the chloroplast Min system at a relatively earlier point of chloroplast evolution than MCD1 (Fig. 1).

Future Perspectives on Evolutionary and Comparative Analyses of the Min System

Although the experimental data are still limited in cyanobacteria and chloroplasts, the differences in composition of the Min

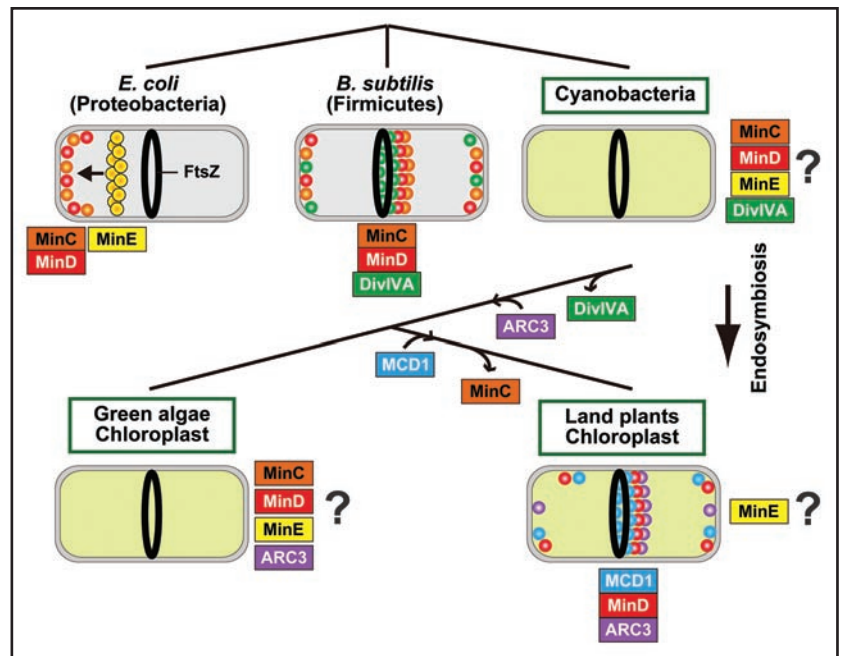


Figure 1. Composition of the Min system and localization of respective components in bacteria and chloroplasts. Only components of known localization are shown inside the cells or chloroplasts. See the text for details.

system among bacteria, plant and algal chloroplasts suggest the existence of several different mechanisms to position the FtsZ ring. In addition, a comparison of the genome sequences along with the identification and characterization of ARC3,^{24,25} MinD^{21,29} and MCD1,⁵ indicate that the chloroplast Min system has undergone stepwise reconstruction by the loss and acquisition of various components (Fig. 1).

For a better understanding the evolution of the chloroplast Min system, (1) information on action of the cyanobacterial Min system is indispensable. In *A. thaliana* chloroplasts, MinD localizes at the division site, as in *B. subtilis* (Fig. 1).⁵ Together with the evident involvement of DivIVA in cyanobacteria as in *B. subtilis*, this suggests that cyanobacterial MinCD localizes at the division site and cell poles, as do MinCD in *B. subtilis*. However, MinE is involved in cyanobacterial and chloroplast division, unlike the case in *B. subtilis*. (2) The localization of MinE in cyanobacteria and chloroplasts will provide critical insights into an understanding of the divergence of the Min system. During the course of chloroplast evolution, ARC3 was acquired and the MinC-like protein was lost (Fig. 1). It is also notable that certain bacterial and archaeal species have MinD, but not MinC.³⁰ (3) Functional experiments on the MinC-like protein, and comparison between action of MinC and ARC3, will reveal how Min system works without MinC, which is a division inhibitor therefore an effector of the Min system in bacteria. As above, there are many points that remain to be addressed. Further studies should provide important insights into the commonalities and differences in the bacterial and chloroplast Min systems.

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